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Cables STANMED

Dear Epter and Josh,

Your stream of epistles are a constant source of entertainment to us all. You appear to be having a vigorous and eventful stay.

Martha and I (and Freki) drove to Seattle a few weeks ago, and got most of the problems for our June arrival settled. We've found a large furnished house for the summer (to accomodate any Fair visitors); Martha has signed a teaching contract, and I received my grant. It was cut somewhat, especially the first year, to a total of $50,000 for the 3 years. NSF approved it, but has no funds now to take up the NIH slack. Perhaps they will be able in the new fiscal year. Actually, I'm pretty well off, and will have access to any equipment I will need. Only Freki was disappointed with the trip. He got to stay in a kennal near Seattle, which didn't suit his idea of a vocation.

The research is building up to a lengthy program in Enzymology, I believe. I do not have the repeat experiment of the labeling by serine-3-C^{14} yet (it will be run tomorrow), but there seems to be no doubt that *B. subtilis* has an active tryp. synthetase. I can routinely demonstrate high activity in whole cell suspensions (higher than wild type *E. coli*), but the activity is lost when the cells are broken up. The secret to showing activity is to use a very low level of tryptophan for growth, since the enzyme is totally repressed, by 5 ug/ml., and 2 ug/ml. give cells with only low activity. The 3 carbon moiety is specifically serine, and the product formed is tryptophan.

I've also prepared C^{14} Ø alanine labeled cells protein, and should have an analysis on this before you get back.

The most suprising aspect of the research concerns the his sensitive (his_S) locus. All 3 of our his_S strains (all recombinable) are also sensitive to Ø alanine. The mapping of this locus is not complete yet, but it appears to be to the right of tyrosine. It definitely is not at the $5MT_R$ locus, which appears to be probably to the left of the aro_2 (shk_1) locus. I've started to analyze the growth characteristics of the his_S mutants in terms of colony size of ~~tyr~~ high dilutions of cells on the appropriate media. In the first experiment of this kind, the ratio of the colony sizes on the different media (after 24 hr.) are approximately:

Ø al + tyr	- 10
Ø al	- 0
Ø al + ~~tyr~~ his	- 0
tyr	- 6
tyr + his	- 6
D.O.	- 8
his	- 0

The Ø al + tyr colonies are very clearly the largest with all 3 strains. Surprisingly colonies are larger on D.O. than on D.O. + tyrosine, which does not fit in well with the (in liquid) growth studies. The age of the plates are not comparable however (tyrosine were the oldest) and this may account for it. I'm repeating it with freshly poured plates, and will also check the growth of SB 32 on his + Ø al. SB 19 (in liquid cultures) was not inhibited appreciably by any amino acid (except slightly by D-L serine, and threonine), and Ø alanine, a tyr, did not stimulate growth

In this connection, you may recall that we have a strain (several strains in fact) that grow well on either histidine or Ø alanine (and are leaky on D.O.). When the following cross is performed, the results are as follows:

32 Su ⟶ × 168

Selecting on Ø al + his

of ~~100~~ 99 colonies picked -

$\frac{74}{100}$ - growth on his, Ø al, but not D.O. (Donor class) (his⁻ Su⁺)

$\frac{21}{100}$ - his⁺ Su⁺ ~~tyr~~ - grow on D.O.

$\frac{4}{100}$ - his⁺ Su⁻ - grow on his, but not Ø al. (32 × 168 gives no colonies able to grow on Ø al.)

Obviously, the present concept is that a suppressor, closely linked, but separable from the his_2^- locus is responsible for the new genotype in SB 32. Whether there is any relationship between this phenomenon and the his_5 locus is obscure at the present time.

I think that further work on these problems must revolve around a study of the enzymatic reactions of histidine and aromatic amino acid biosynthesis. I'm particularly interested in the effect of histidine on prephenic dehydrogenase and the transaminases. It will also be interesting to determine whether we can detect enzymatic activity of other type enzymes in extracts.

We're also actively working on the kinetics of competency, penicillin killing of transformants, and # Nothing of interest to report on these studies.

I'm looking forward to your arrival at Stanford - to discuss your trip and the numerous ideas that have developed from it.

Sincerely,

Gene

P. S. Martha sends her best regards.